EAST Search History

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	644	Granzyme adj B	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:50
L2	205	Hsp70 near7 tumor	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:50
L3	114	Hsp70 near7 cancer	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:50
L4	26	Hsp70 near7 carcino$	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:51
L5	4	Hsp70 near7 neoplas$	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:53
L6	185	L1 and (NK adj cell)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:53
L7	48	L2 and (NK adj cell)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:53
L8	18	L3 and (NK adj cell)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:58
L9	8	L4 and (NK adj cell)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:55
L10	1	L5 and (NK adj cell)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 15:08
L11	52	L1 and hsp70	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:58
L12	12	L11 and @pd<="20030822"	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 15:00
L13	2	L1 and L2	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 14:59
L14	0	L1 and (granzyme same administer)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 15:09
L15	67	L1 and (granzyme same patient)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 15:16

EAST Search History

L16	57	L1 and (ion adj channel)	US-PGPUB; USPAT; USOCR	OR	ON	2007/01/08 15:16

US 20060216707 A1 US-PGPUB
US 20060120997 A1 US-PGPUB
US 20060111285 A1 US-PGPUB
US 20060073589 A1 US-PGPUB
US 20050008649 A1 US-PGPUB
US 20040063173 A1 US-PGPUB
US 7132109 B1 USPAT
US 6984389 B2 USPAT

US 20030148511 A1 US-PGPUB
US 20030148316 A1 US-PGPUB
US 20030134302 A1 US-PGPUB
US 20030073163 A1 US-PGPUB
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US 20020086844 A1 US-PGPUB
US 20020009730 A1 US-PGPUB
US 20010011078 A1 US-PGPUB
US 6544523 B1 USPAT
US 6335170 B1 USPAT
US 6221355 B1 USPAT
US 6165737 A USPAT

PALM INTRANET

Day : Monday
Date: 1/8/2007
Time: 15:51:47

Inventor Name Search Result

Your Search was:

Last Name = MULTHOFF
First Name = G

Application#	Patent#	Status	Date Filed	Title	Inventor Name
08970699	5932478	150	11/14/1997	HUMAN COLON CARCINOMA CELL LINES SHOWING STABLE HSP72 EXPRESSION	MULTHOFF, GABRIELE
09273616	6261839	250	03/22/1999	METHOD FOR THE INDUCTION OF A NK CELL-MEDIATED IMMUNE RESPONSE	MULTHOFF, GABRIELE
09646835	Not Issued	41	01/11/2001	Use of hsp70 proteins	MULTHOFF, GABRIELE
10380408	Not Issued	41	08/25/2003	Hsp70 peptide stimulating natural killer (nk) cell activity , and uses thereof	MULTHOFF, GABRIELE
10526586	Not Issued	71	12/12/2005	Use of granzyme b as an hsp70/hsp70 peptide dependent inducer of apoptosis in tumor cells	MULTHOFF, GABRIELE
10581960	Not Issued	19	01/01/0001	Therapeutic and diagnostic anti-hsp 70 antibodies	MULTHOFF, GABRIELE

Inventor Search Completed: No Records to Display.

Search Another: Inventor Last Name: multhoff First Name: g Search

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multimmune

Company Profile

multimmune GmbH is a biopharmaceutical comp discovery and development of novel products for tl and infectious diseases through its innovative mani system.

The company was founded in August 1999 by Prof and Dr. Claus Botzler because of the very prom innovative method that enables for the first time activation of **Natural Killer (NK) cells** a major b therapy.

The company actually focuses on the development to treat heat shock protein 70 (Hsp70) positive t Hsp70 is a tumor-specific marker expressed on ab cancer entities, e.g. lung, colon, breast.

Four approaches are being explored to destroy canc

- by extracorporal activation of Natural Killer (NI peptide mimicking Hsp70 (ENKASTIM, ready to go into phase II)
- by direct infusion of the Hsp70 analogue (prec
- by using a tumor-specific antibody against Hs (mi-TUMEX; R&D stage)
- by inducing tumor-specific apoptosis via Hsp7((mi-APO; R&D stage)

Safety of treatment, biological and first clinical effica ENKASTIM, was proven in a phase I clinical pilot stu colon and lung cancer.

Business Model

Our business is to design and develop innovative c

of cancer. The company is building a broad pipeline the basis of proprietary technologies.

Creating value:

- pipeline of drug candidates
- product and technology platform based on re Natural Killer (NK) cells

Commercialization:

- outlicensing of drug candidates to pharma an biotech companies, typically on the basis of ph
- selected co-development partnerships technologies

Patents

Patents in the fields of Heat Shock Proteins and NK

Partners

- University Hospitals of Regensburg and Munic
- research institutions

Management

Gabriele Multhoff, Ph.D.,
Professor for Molecular Oncology; CEO/CSO

Gabriele is co-founder of multimmune and also hc the University Hospital of Regensburg. After re immunology (1991) from the Institute of Immunolog she headed the research group "Heat Sho GSF/University Hospital Grosshadern. In 1998 s doctorate (Habilitation: "Characterization of therap in tumor and effector cells: an in vitro model for t adjuvant immunotherapy") and since 2003 she "Consultant Immunologist".

Claus Botzler, Ph.D., MBA
CEO/CFO (finance & administration, business devel

Claus is co-founder of multimmune and studied bio of Freiburg and Munich. He received his Ph.D. in

1992 (Thesis: Expression of Hsp70 on tumor cel
was working in the field of Heat shock proteins a
post-doctoral fellow at the GSF and the University
In 2003 he gained a MBA degree at the Open Univ
(Milton Keynes, GB).

Scientific advisory board

Prof. Dr. R. Andreesen (University Hospital Regenst
Prof. Dr. A. Asea (Boston University School of Medi
Prof. Dr. H-J. Feldmann (Hospital for Radiooncology
Prof. Dr. W. Hiddemann (University Hospital Munich
Prof. Dr. L. Hightower (University of Connecticut)
Prof. Dr. D. Schendel (GSF Munich)
Prof. A. Velardi (University Hospital Perugia)

Finance

We are financed by private and institutional ir
BayernKapital) and by public grants (BMBF)
multimmune has raised about 3.6 million Euro.

Premises

multimmune is located at the University Hospital
direct neighbourhood of the University Regensb
Regensburg.

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"author:G. author:Multhoff" Search

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G Multhoff
C Botzler
R Issels
G Eissner
M Wiesnet

Heat shock protein 72 on tumor cells: a recognition structure for natural killer cells - group of 3 »
G Multhoff - The Journal of Immunology, 1997 - Am Assoc Immnol
Evidence is accumulating that members of the heat shock protein 70 (HSP70) family are found on the cell surface of certain tumor cells where they ...
Cited by 128 - Related Articles - Web Search - BL Direct

A stress-inducible 72-kDa heat-shock protein (HSP72) is expressed on the surface of human tumor ... - group of 3 »
G Multhoff, C Botzler, M Wiesnet, E Muller, T ... - Int J Cancer, 1995 - ncbi.nlm.nih.gov
It is suggested that members of the heat-shock protein (HSP) 70 and 90 families are involved in intracellular antigen processing and the presentation of ...
Cited by 113 - Related Articles - Web Search - BL Direct

Critical involvement of transmembrane tumor necrosis factor-alpha in endothelial programmed cell ... - group of 3 »
... , P Scheurich, A Hieke, **G Multhoff**, GW Bornkamm, E ... - Blood - Am Soc Hematology
POPTOTIC PROGRAMMED cell death occurs under physiologic and pathophysiologic conditions. In the immune system, apoptosis serve for selection of B cells ...
Cited by 87 - Related Articles - Web Search - BL Direct

Heat shock protein 70 (Hsp70) stimulates proliferation and cytolytic activity of natural killer ... - group of 3 »
G Multhoff, L Mizzen, CC Winchester, CM Milner, S ... - Exp Hematol, 1999 - ncbi.nlm.nih.gov
We previously demonstrated that lysis of tumor cells that express Hsp70, the highly stress-inducible member of the HSP70 family, on their plasma ...
Cited by 70 - Related Articles - Web Search - BL Direct

Peripheral Blood Mononuclear Cells Induce Programmed Cell Death in Human Endothelial Cells and May ... - group of 3 »
... , E Holler, B Ertl, **G Multhoff**, M Schreglmann, I ... - Blood, 1997 - Am Soc Hematology
Human umbilical vein endothelial cells (HUVECs) undergo programmed cell death (apoptosis) after coculture with peripheral blood mononuclear cells (PBMCs) ...
Cited by 59 - Related Articles - Web Search - BL Direct

CD3-large granular lymphocytes recognize a heat-inducible immunogenic determinant associated with ... - group of 3 »
G Multhoff, C Botzler, M Wiesnet, G Eissner, R ... - Blood, 1995 - Am Soc Hematology
Traditionally, heat shock proteins (HSPs) are believed to be located intracellularly, where they perform a variety of chap- eroning functions. ...
Cited by 56 - Related Articles - Web Search - BL Direct

Definition of extracellular localized epitopes of Hsp70 involved in an NK immune response - group of 5 »
C Botzler, G Li, RD Issels, **G Multhoff** - Cell Stress & Chaperones - cest.allenpress.com
In order to define extracellular localized epitopes of Hsp70 on human tumor cells which are accessible to the immune system, six commercially available ...

Cited by 53 - Related Articles - Web Search - BL Direct

Cell Surface Expression of Heat Shock Proteins and the Immune Response - group of 4 »
G Multhoff, LE Hightower - Cell Stress & Chaperones, 1996 - JSTOR
Cell Surface Expression of Heat Shock Proteins and the Immune Response. Gabriele Multhoff. Lawrence E. Hightower. Cell Stress & Chaperones, Vol. 1, No. ...
Cited by 48 - Related Articles - Web Search

Heat-shock protein 72 cell-surface expression on human lung carcinoma cells is associated with an ... - group of 3 »
C Botzler, R Issels, **G Multhoff** - Cancer Immunology, Immunotherapy, 1996 - Springer
AbstractmThe cell-surface expression patterns of major histocompatibility complex (MHC) class I, class II and heat-shock protein 72 (HSP72) molecules ...
Cited by 41 - Related Articles - Web Search - BL Direct

The role of heat shock proteins in the stimulation of an immune response. - group of 2 »
G Multhoff, C Botzler, R Issels - Biol Chem, 1998 - ncbi.nlm.nih.gov
Heat shock proteins (HSP) have been defined as immunodominant, although most of them are highly conserved and ubiquitously distributed. Members of the 60, ...
Cited by 39 - Related Articles - Web Search - BL Direct

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"author:G. author:Multhoff" Search

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MULTHOFF 10 526 586 = Granzyme B & Hsp70 vs. tumor/cancer

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L2           29 L1 AND HSP70

=> s L1 and natural killer
L3         1499 L1 AND NATURAL KILLER

=> s L1 and administer granzyme
L4            0 L1 AND ADMINISTER GRANZYME

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L6 ANSWER 1 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 1
AN 2007:31559 BIOSIS
DN PREV200700028705
TI Hop cleavage and function in granzyme B-induced apoptosis.
AU Bredemeyer, Andrew J.; Carrigan, Patricia E.; Fehniger, Todd A.; Smith, David F.; Ley, Timothy J. [Reprint Author]
CS Washington Univ, Sch Med, Dept Med, Div Oncol, Campus Box 8007,660 S Euclid Ave, St Louis, MO 63110 USA
tley@im.wustl.edu
SO Journal of Biological Chemistry, (DEC 1 2006) Vol. 281, No. 48, pp. 37130-37141.
CODEN: JBCHA3. ISSN: 0021-9258.
DT Article
LA English
ED Entered STN: 27 Dec 2006
Last Updated on STN: 27 Dec 2006
AB Granzyme B (GzmB) is a cytotoxic protease found in the granules of natural killer cells and cytotoxic T lymphocytes. GzmB cleaves multiple intracellular protein substrates, leading to caspase activation, DNA fragmentation, cytoskeletal instability, and rapid induction of target cell apoptosis. However, no known individual substrate is required for GzmB to induce apoptosis. GzmB is therefore thought to initiate multiple cell death pathways simultaneously to ensure the death of target cells. We previously identified Hop (Hsp70 /Hsp90-organizing protein) as a GzmB substrate in a proteomic survey (Bredemeyer, A. J., Lewis, R. M., Malone, J. P., Davis, A. E., Gross, J., Townsend, R. R., and Ley, T. J. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 11785 11790). Hop is a co-chaperone for Hsp70 and Hsp90, which have been implicated in the negative regulation of apoptosis. We therefore hypothesized that Hop may have an anti-apoptotic function that is abolished upon cleavage, lowering the threshold for GzmB-induced apoptosis. Here, we show that Hop was cleaved directly by GzmB in vitro and in cells undergoing GzmB induced apoptosis. Expression of the two cleavage fragments of Hop did not induce cell death. Although cleavage of Hop by GzmB destroyed Hop function in vitro, both cells overexpressing GzmB-resistant Hop and cells with a 90-95% reduction in Hop levels exhibited unaltered susceptibility to GzmB-induced death. We conclude that Hop per se does not set the threshold for susceptibility to GzmB-induced apoptosis. Although it is possible that Hop may be cleaved by GzmB as an "innocent bystander" during the induction of apoptosis, it may also act to facilitate apoptosis in concert with other GzmB substrates.

L6 ANSWER 2 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2
AN 2006:599032 BIOSIS
DN PREV200600590545
TI Granzyme B proteolyzes receptors important to proliferation and survival, tipping the balance toward apoptosis.
AU Loeb, Carly R. K.; Harris, Jennifer L.; Craik, Charles S. [Reprint Author]
CS Univ Calif San Francisco, Dept Pharmaceut Chem, MC 2280,Genentech Hall,Rm S512,600 16th St, San Francisco, CA 94131 USA
craik@cgl.ucsf.edu
SO Journal of Biological Chemistry, (SEP 22 2006) Vol. 281, No. 38, pp. 28326-28335.
CODEN: JBCHA3. ISSN: 0021-9258.
DT Article

LA English
ED Entered STN: 8 Nov 2006
Last Updated on STN: 8 Nov 2006
AB Granzyme B is critical to the ability of natural killer cells and cytotoxic T lymphocytes to induce efficient cell death of virally infected or tumor cell targets. Although granzyme B can cleave and activate caspases to induce apoptosis, granzyme B can also cause caspase-independent cell death. Thirteen prospective granzyme B substrates were identified from a cDNA expression-cleavage screen, including Hsp70, Notch1, fibroblast growth factor receptor-1 (FGFR1), poly-A-binding protein, cAbl, heterogeneous nuclear ribonucleoprotein H', Br140, and intersectin-1. Validation revealed that Notch1 is a substrate of both granzyme B and caspases, whereas FGFR1 is a caspase-independent substrate of granzyme B. Proteolysis of FGFR1 in prostate cancer cells has functionally relevant consequences that indicate its cleavage may be advantageous for granzyme B to kill prostate cancer cells. Therefore, granzyme B not only activates pro-death functions within a target, but also has a previously unidentified role in inactivating pro-growth signals to cause cell death.

L6 ANSWER 3 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 3
AN 2006:680469 BIOSIS
DN PREV200600666242
TI Proteolysis of HIP during apoptosis occurs within a region similar to the BID loop.
AU Caruso, Joseph A. [Reprint Author]; Reiners, John J. Jr.
CS Wayne State Univ, Inst Environm Hlth Sci, 2727 2nd Ave,Rm 4000, Detroit, MI 48201 USA
Joseph_Caruso@wayne.edu
SO Apoptosis, (NOV 2006) Vol. 11, No. 11, pp. 1877-1885.
ISSN: 1360-8185.
DT Article
LA English
ED Entered STN: 6 Dec 2006
Last Updated on STN: 6 Dec 2006
AB BID is an essential component of many apoptotic pathways. Cytosolic proteases cleave BID within an extended loop region, generating an active truncated fragment which synergizes with BAX and BAK to induce release of apoptogenic factors from mitochondria. To determine whether other proteins are cleaved in a similar manner as BID, we performed a database search for proteins which possess sequence similarity with the BID loop region. One of the proteins identified was the Hsc70-interacting protein (HIP). We analyzed the cleavage pattern of HIP using two known activators of BID: granzyme B and caspase-8. In in vitro cleavage assays using recombinant proteins, human and rat HIP were cleaved by granzyme B. Furthermore, the granzyme B-mediated cleavage site was mapped to the BID loop-like region of HIP by site-directed mutagenesis. This region was also the target for caspase-8-mediated cleavage in rat HIP. However, human HIP was not proteolyzed by caspase-8, which probably reflects sequence differences between human and rat HIP proteins at the P-1' position of the caspase-8 recognition sequence. To determine whether HIP is cleaved during apoptosis, human Jurkat T cells were exposed to granzyme B and perforin. The results of these studies suggest that granzyme B-mediated loss of HIP expression occurs in vivo, and in a coordinate fashion with loss of BID, pro-caspase-8 and pro-caspase-3. These data implicate the Hsp70 co-chaperone HIP in the proteolytic cascade of some apoptotic pathways.

L6 ANSWER 4 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

DUPLICATE 4
AN 2005:362246 BIOSIS
DN PREV200510146115
TI Heat shock protein 70 surface-positive tumor exosomes stimulate migratory and cytolytic activity of natural killer cells.
AU Gastpar, Robert; Gehrmann, Mathias; Bausero, Maria A.; Asea, Alexzander; Gross, Catharina; Schroeder, Josef A.; Multhoff, Gabriele [Reprint Author]
CS Univ Hosp Regensburg, Dept Hematol and Oncol, Franz Josef Strauss Allee 11, D-93053 Regensburg, Germany
gabriele.multhoff@klinik.uni-regensburg.de
SO Cancer Research, (JUN 15 2005) Vol. 65, No. 12, pp. 5238-5247.
CODEN: CNREA8. ISSN: 0008-5472.
DT Article
LA English
ED Entered STN: 14 Sep 2005
Last Updated on STN: 14 Sep 2005
AB Detergent-soluble membrane vesicles are actively released by human pancreas (Colo-/Colo+) and colon (CX-/CX+) carcinoma sublines, differing in their capacity to present heat shock protein 70 (Hsp70)/Bag-4 on their plasma membranes. Floating properties, acetylcholine esterase activity, and protein composition characterized them as exosomes. An enrichment of Rab-4 documented their intracellular transport route from early endosomes to the plasma membrane. After solubilization, comparable amounts of cytosolic proteins, including tubulin, Hsp70, Hsc70, and Bag-4, but not ER-residing Grp94 and calnexin, were detectable in tumor-derived exosomes. However, with respect to the exosomal surface, only Colo+/CX+ but not Colo-/CX- derived exosomes were Hsp70 membrane positive. Therefore, concomitant with an up-regulated cell surface density of activation markers, migration and Hsp70 reactivity of natural killer (NK) cells was stimulated selectively by Hsp70/Bag-4 surface-positive exosomes, but not by their negative counterparts and tumor cell lysates. Moreover, the exosome-mediated lytic activity of NK cells was blockable by Hsp70-specific antibody. As already shown for TKD stimulation, NK cells preincubated with Hsp70 surface-positive exosomes initiated apoptosis in tumors through granzyme B release. In summary, our data provide an explanation how Hsp70 reactivity in NK cells is induced by tumor-derived exosomes.

L6 ANSWER 5 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 5
AN 2005:538011 BIOSIS
DN PREV200510329603
TI Nucleofection of non-B cells with mini-Epstein-Barr virus DNA.
AU Radons, Juergen; Gross, Catharina; Stangl, Stefan; Multhoff, Gabriele [Reprint Author]
CS Univ Regensburg, Abt Hamatol and Internist Onkol, Franz Josef Str Allee 11, D-93053 Regensburg, Germany
gabriele.multhoff@klinik.uni-regensburg.de
SO Journal of Immunological Methods, (AUG 2005) Vol. 303, No. 1-2, pp. 135-141.
CODEN: JIMMBG. ISSN: 0022-1759.
DT Article
LA English
ED Entered STN: 1 Dec 2005
Last Updated on STN: 1 Dec 2005
AB A tumor-specific cell surface localization of heat shock protein 70 (Hsp70) on CX+ colon carcinoma cells provides a recognition structure for NK cells but not for NKT and T cells. Incubation with low-dose IL-2 plus Hsp70-peptide TKD enhances production and release of granzyme B by NK cells and thus renders Hsp70-positive tumors more sensitive to their cytolytic attack. To provide the experimental basis for the generation of Hsp70

-reactive NK cell lines we established a modified nucleofection technique as a rapid and efficient method for gene transfer into non-B cells. Therefore, TKD-stimulated, CD3/CD19-depleted effector cells, consisting of 85% CD3(-) CD16/56(+) NK cells, 1.4% CD3(+) CD16/56+ NKT cells, and 0.3% CD3(+) CD16/56(-) T cells were nucleofected with the green fluorescent protein (GFP)-containing mini-Epstein-Barr virus (mini-EBV) plasmid p2667 (1478.A d2GFP). GFP, a marker for the expression of EBV-associated genes, became visible for the first time on day 18 after transfection. On day 28 mini-EBV-transfected cells consisted of 49% NKT, 38% T cells, and 13% NK cells; no contaminating B cells were detectable. Even 1.5 years after transfection GFP and CD94 were found to be co-expressed on transfectants. These data indicated that mini-EBV provides a useful tool for the nucleofection of non-B cells. The cytolytic activity of NK-transfectants towards Hsp70 membrane-positive CX+ tumor cells was comparable to that of non-transfected effector cells. In summary, our results might provide the basis for the generation of non-B effector cell lines including NK cells with conserved Hsp70-reactivity.

L6 ANSWER 6 OF 10 MEDLINE on STN
AN 2004273335 MEDLINE
DN PubMed ID: 15173076
TI Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: a clinical phase i trial.
AU Krause Stefan W; Gastpar Robert; Andreesen Reinhard; Gross Catharina; Ullrich Heidrun; Thonigs Gerald; Pfister Karin; Multhoff Gabriele
CS Department of Hematology/Oncology, Institute for Clinical Chemistry, University Hospital Regensburg, Regensburg, Germany.
SO Clinical cancer research : an official journal of the American Association for Cancer Research, (2004 Jun 1) Vol. 10, No. 11, pp. 3699-707. Journal code: 9502500. ISSN: 1078-0432.
CY United States
DT (CLINICAL TRIAL)
(CLINICAL TRIAL, PHASE I)
Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200412
ED Entered STN: 3 Jun 2004
Last Updated on STN: 28 Dec 2004
Entered Medline: 27 Dec 2004
AB PURPOSE: The 14 amino acid sequence (aa(450-463)) TKDNNLLGRFELSG (TKD) of heat shock protein 70 (Hsp70) was identified as a tumor-selective recognition structure for natural killer (NK) cells. Incubation of peripheral blood lymphocyte cells with TKD plus low-dose interleukin 2 (IL-2) enhances the cytolytic activity of NK cells against Hsp70 membrane-positive tumors, in vitro and in vivo. These data encouraged us to test tolerability, feasibility, and safety of TKD-activated NK cells in a clinical Phase I trial. EXPERIMENTAL DESIGN: Patients with metastatic colorectal cancer (n = 11) and non-small cell lung cancer (n = 1) who had failed standard therapies were enrolled. After ex vivo stimulation of autologous peripheral blood lymphocytes with Hsp70-peptide TKD (2 microg/ml) plus low-dose IL-2 (100 units/ml), TKD was removed by extensive washing, and activated cells were reinfused i.v. The procedure was repeated for up to six cycles, applying a dose escalation schedule in 4 patients. RESULTS: The percentage of activated NK cells in the reinfused leukapheresis products ranged between 8 and 20% of total lymphocytes, corresponding to total NK cell counts of 0.1 up to 1.5 x 10(9). Apart from restless feeling in 1 patient and itching in 2 patients, no negative side effects were observed. Concomitant with an enhanced CD94 cell surface density, the cytolytic activity of NK cells against Hsp70 membrane-positive colon carcinoma cells was

enhanced after TKD/IL-2 stimulation in 10 of 12 patients. Concerning tumor response, 1 patient was in stable disease during therapy by formal staging criteria and another patient showed stable disease in one metastases and progression in another. CONCLUSIONS: Reinfusion of Hsp70-activated autologous NK cells is safe. Immunological results warrant additional studies in patients with lower tumor burden.

L6 ANSWER 7 OF 10 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 6
AN 2004233364 EMBASE
TI Differential up-regulation of cytosolic and membrane-bound heat shock protein 70 in tumor cells by anti-inflammatory drugs.
AU Gehrmann M.; Brunner M.; Pfister K.; Reichle A.; Kremmer E.; Multhoff G.
CS G. Multhoff, Dept. of Hematology and Oncology, University Hospital Regensburg, Franz-Josef-Strauss Allee 11, 93053 Regensburg, Germany. gabriele.multhoff@klinik.uni-regensburg.de
SO Clinical Cancer Research, (15 May 2004) Vol. 10, No. 10, pp. 3354-3364. . Refs: 40
ISSN: 1078-0432 CODEN: CCREF4
CY United States
DT Journal; Article
FS 016 Cancer
030 Pharmacology
037 Drug Literature Index
LA English
SL English
ED Entered STN: 28 Jun 2004
Last Updated on STN: 28 Jun 2004
AB Purpose: Modulation of the heat shock protein (HSP) response affects sensitivity to therapeutic agents in cancer. Here, drugs with anti-inflammatory potential (cyclooxygenase 1/2 inhibitors) and peroxidase proliferator-activated receptor-.gamma. agonists were analyzed for their capacity to affect Hsp70 expression in human cancer cells with a divergent Hsp70 membrane expression pattern. Experimental Design: In dose kinetics, the nonlethal concentration of acetyl-salicyl acid, celecoxib, rofecoxib, and the insulin-sensitizer pioglitazone was identified for the human adenocarcinoma cell line CX-. With the exception of CLX, which was diluted in DMSO, all reagents were dissolved in water. After treatment with the different compounds at nontoxic concentrations for 6 h, followed by a 1-h recovery period, the cytosolic Hsp70 levels were measured in CX-2 and CX- tumor cells by Western blot analysis. Fold increase was calculated in relation to the housekeeping protein tubulin. Membrane-bound Hsp70 was analyzed by flow cytometry using a FITC-labeled Hsp70-specific monoclonal antibody. Untreated cells and cells incubated with equivalent amounts of the diluting agents served as controls. The immunological function was tested in granzyme B apoptosis assays, standard (51)Cr release assays, and antibody blocking studies. Results: Compared with aqua dest, the cytoplasmic amount of Hsp70 was equally enhanced in CX-2 and CX-cells by all compounds. An increase in membrane-bound Hsp70, detected selectively in CX- cells, corresponded to an enhanced sensitivity to granzyme B- and natural killer cell-mediated kill that was blockable by using a Hsp70-specific antibody. Conclusions: Although increase in cytosolic Hsp70 levels conferred resistance to further stress, membrane-bound Hsp70 rendered tumor cells more sensitive to the immunological attack mediated by granzyme B and natural killer cells. Our data provide a biological rational for combining anti-inflammatory drugs with immunotherapy in cancer therapy.

L6 ANSWER 8 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
AN 2005:479312 BIOSIS
DN PREV200510271216

TI Development and function of NK and T cells in AML patients after allogeneic stem cell transplantation (SCT).

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CS Univ Regensburg, Dept Hematol and Oncol, D-8400 Regensburg, Germany

SO Blood, (NOV 16 2004) Vol. 104, No. 11, Part 1, pp. 886A.
Meeting Info.: 46th Annual Meeting of the American-Society-of-Hematology. San Diego, CA, USA. December 04 -07, 2004. Amer Soc Hematol.
CODEN: BLOOAW. ISSN: 0006-4971.

DT Conference; (Meeting)
Conference; (Meeting Poster)

LA English

ED Entered STN: 16 Nov 2005
Last Updated on STN: 16 Nov 2005

AB Purpose: Hsp70 was frequently found on the plasma membrane of bone marrow-derived leukemic blasts, but not on normal bone marrow cells. Hsp70 membrane expression could be correlated with protection against therapy-induced apoptosis (Nylandsted et al 2004). In contrast, these tumor cells have been found to be highly sensitive to the cytolytic attack mediated by NK cells. In vitro, Hsp70-activated NK cells efficiently lysed autologous Hsp70 membrane-positive leukemic blasts (Gehrmann et al 2003). Granzyme B release served as a surrogate marker for estimating the cytolytic response of NK cells against Hsp70 membrane-positive tumor target cells (Gross et al 2003). Here, we studied the development of NK and T cells in AML patients (n=6) after allogeneic SCT at different timepoints (days 14-20, 45, 90, 180, 1 year) after allogeneic stem cell transplantation (SCT).Methods: HLA class I, HLA-E and Hsp70 surface expression was determined on allpatient-derived leukemic blasts of the bone marrow by flow cytometry. The amount of NK and T cells was investigated by multicolor flow cytometry using CD3/CD16 and CD56 and CD94/CD56 antibody-combinations detecting NK cell specific markers. Effector cell function was tested in a granzyme B ELISPOT assay against patient-derived leukemic blasts and K562 cells.Results: All tested leukemic blasts were positive for HLA class 1, HLA-E, and Hsp70. After induction therapy the amount of CD3-negative, CD56/CD94-positive NK cells was 28 +/- 16%, that of CD3-positive T cells was 58 +/- 3%. On days 14-21 after allogeneic SCT, 58 +/- 9% of the donor-derived peripheral blood lymphocytes (PBL) were CD3-negative, CD56/CD94-positive NK cells; the amount of CD3-positive T cells was 26 +/- 7.5%. On day 45, the amount of NK cells furtherincreased up to 68 +/- 7.9%; that of T cells further decreased down to 16 +/-5.6%. On day 90 and day 180 the amount of NK cells was still 41 +/- 10%; thatof T cells was 29 +/- 12%. Interestingly, high NK cell counts correlated withan increased cytolytic response against leukemic blast and K562 cells.One year after allogeneic, SCT, NK (20 +/- 1%) and T cell (52 +/- 18%) ratios were comparable to that of healthy human individuals.Conclusions: Between days 14 and 180 after allogeneic SCT, the amount of NK cells was significantly elevated if compared to that of T cells. Concomitantly, cytolytic function against leukemic blasts was significantly elevated. Normal levels, in the composition of NK and T cells were reached I year after SCT.

L6 ANSWER 9 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN

AN 2004:95218 BIOSIS

DN PREV200400084222

TI Enhancement of DNA vaccine potency by coadministration of a tumor antigen gene and DNA encoding serine protease inhibitor-6.

AU Kim, Tae Woo; Hung, Chien-Fu; Boyd, David A. K.; He, Liangmei; Lin, Cheng-Tao; Kaiserman, Dion; Bird, Phillip I.; Wu, T.-C. [Reprint Author]

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SO Cancer Research, (January 1 2004) Vol. 64, No. 1, pp. 400-405. print.
ISSN: 0008-5472 (ISSN print).
DT Article
LA English
ED Entered STN: 11 Feb 2004
Last Updated on STN: 11 Feb 2004
AB Serine protease inhibitor 6 (SPI-6), also called Serpinb9, inhibits granzyme B and thus may provide a method for delaying apoptotic cell death in dendritic cells. We have previously enhanced DNA vaccine potency by targeting antigen to MHC antigen presentation pathways, using proteins such as Mycobacterium tuberculosis heat shock protein 70, calreticulin, domain II of Pseudomonas aeruginosa exotoxin A, or the sorting signal of the lysosome-associated membrane protein type 1. In this study, we explored intradermal coadministration of DNA encoding SPI-6 with DNA constructs encoding human papillomavirus type 16 E7 linked to these intracellular targeting molecules for its ability to generate E7-specific CD8+ T-cell immune responses and E7-specific antitumor effects. This combination of strategies resulted in significantly increased E7-specific CD8+ T-cell and CD4+ Th1-cell responses, enhanced tumor treatment ability, and stronger tumor protection when compared with vaccination without SPI-6. Among these targeting strategies tested, mice vaccinated with Sig/E7/lysosome-associated membrane protein type 1 mixed with SPI-6 showed the greatest fold increase in E7-specific CD8+ T cells (apprx5-fold). Vaccination with a nonfunctional mutant of SPI-6 did not result in immune enhancement, indicating that enhancement was dependent on the antiapoptotic function of SPI-6. Our results suggest that DNA vaccines combining strategies that enhance MHC class I and II antigen processing with SPI-6 have potential clinical implications for control of viral infection and neoplasia.

L6 ANSWER 10 OF 10 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 7
AN 2003:577657 BIOSIS
DN PREV200300583456
TI Cell surface-bound heat shock protein 70 (Hsp70) mediates perforin-independent apoptosis by specific binding and uptake of granzyme B.
AU Gross, Catharina; Koelch, Walter; DeMaio, Antonio; Arispe, Nelson; Multhoff, Gabriele [Reprint Author]
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gabriele.multhoff@klinik.uni-regensburg.de
SO Journal of Biological Chemistry, (October 17 2003) Vol. 278, No. 42, pp. 41173-41181. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DT Article
LA English
ED Entered STN: 10 Dec 2003
Last Updated on STN: 10 Dec 2003
AB Cell surface-bound heat shock protein 70 (Hsp70) renders tumor cells more sensitive to the cytolytic attack mediated by natural killer (NK) cells. A 14-amino acid Hsp70 sequence, termed TKD (TKDNNLLGRFELSG, aa450-463) could be identified as the extracellular localized recognition site for NK cells. Here, we show by affinity chromatography that both, full-length Hsp70-protein and Hsp70-peptide TKD, specifically bind a 32-kDa protein derived from NK cell lysates. The serine protease granzyme B was uncovered as the 32-kDa Hsp70-interacting protein using matrix-assisted laser desorption ionization time-of-flight mass peptide fingerprinting. Incubation of tumor cells with increasing concentrations of perform-free, isolated granzyme B shows specific binding and uptake in a dose-dependent manner and results in initiation of apoptosis selectively in tumor cells presenting Hsp70 on the

cell surface. Remarkably, Hsp70 cation channel activity was also determined selectively in purified phospholipid membranes of Hsp70 membrane-positive but not in membrane-negative tumor cells. The physiological role of our findings was demonstrated in primary NK cells showing elevated cytoplasmic granzyme B levels following contact with TKD. Furthermore, an increased lytic activity of Hsp70 membrane-positive tumor cells could be associated with granzyme B release by NK cells. Taken together we propose a novel perform-independent, granzyme B -mediated apoptosis pathway for Hsp70 membrane-positive tumor cells.

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=> s L1 and administer(P)granzyme
L7            0 L1 AND ADMINISTER(P) GRANZYME

=> s L1 and adminiister(P)natural killer
L8            0 L1 AND ADMINIISTER(P) NATURAL KILLER
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